4.0 ICCVAM Consideration of Public and SACATM Comments

ICCVAM received 27 public comments in response to four *FR* notices released between May 2007 and May 2008 (see **Appendix G**). Comments received in response to or related to the *FR* notices are also available on the NICEATM–ICCVAM website.[21] The following sections, delineated by *FR* notice, briefly discuss the public comments received.

4.1 Public Comments in Response to 72 FR 27815 (May 17, 2007): The Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data

NICEATM requested the following:

1. Public comments on the appropriateness and relative priority of evaluation of the validation status of
    a. The LLNA as a stand-alone assay for determining potency (including severity) for the purpose of hazard classification
    b. The rLLNA approach
    c. Non-radioactive LLNA methods
    d. The use of the LLNA for testing mixtures, aqueous solutions, and metals
    e. The current applicability domain
2. Nominations of expert scientists to consider as members of a possible peer review panel
3. Submission of data for the LLNA and/or modified versions of the LLNA

In response to this *FR* notice, NICEATM received 17 comments. Six comments included additional data and information, while two others offered data and information upon request. Three nominated four potential panelists for consideration. Three commenters suggested reference publications for consideration during the Panel evaluation. NICEATM provided the data and suggested references to the Panel for evaluation.

Three comments remarked specifically on the rLLNA.

One commenter suggested rearranging the priority sequence of test method evaluation from most to least pressing: a, e, d, b, and c (see list above). ICCVAM did not establish a relative priority for these activities because they were all considered to be high-priority activities. Accordingly, all LLNA-related activities described above were discussed at the March 2008 Panel meeting.

Another commenter noted that ESAC issued a statement supporting the use of the rLLNA "within tiered-testing strategies to reliably distinguish between chemicals that are skin sensitisers and non-sensitisers" (**Appendix E**), thereby reducing animal use by as much as 50%. The ESAC statement also notes the following limitations: "the test results provided by the rLLNA do not allow the determination of the potency of a sensitising chemical" and "negative

[21] Available at http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm

test results associated with testing using concentrations of less than 10% should undergo further evaluation." The commenter states that ICCVAM should (1) expeditiously review and endorse the ESAC peer review and circulate harmonized testing recommendations regarding this assay to U.S. agencies before the end of the year, and (2) NICEATM should collaborate with ECVAM to address the question of concentration threshold.

As indicated in **Section 1.0**, ICCVAM and NICEATM collaborated with liaisons from ECVAM and JaCVAM to update with 260 additional LLNA studies the Kimber et al. (2006) evaluation upon which the ESAC statement was based. This comprehensive evaluation was expedited for inclusion in the publicly transparent ICCVAM peer review process, which included the March 2008 Panel meeting.

A third commenter stated that ESAC considered the rLLNA to be scientifically validated but only when used as a screening test to distinguish between sensitizers and non-sensitizers and with due regard to the conditions set forth in the official ESAC statement of April 27, 2007. This statement was based on the outcome of a review of LLNA data for 211 chemicals (Kimber et al. 2006). The review of existing and newly provided LLNA data proposed by NICEATM–ICCVAM therefore presents an ideal opportunity to assess further the validity of the rLLNA for screening purposes. The ICCVAM test method recommendations detailed in **Section 2.0** describe the usefulness and limitations of the rLLNA based on the comprehensive ICCVAM evaluation of an expanded database of 471 LLNA studies.

4.2 Public Comments in Response to 72 FR 52130 (September 12, 2007): Draft Performance Standards for the Murine Local Lymph Node Assay: Request for Comments

NICEATM requested public comments on the initial ICCVAM-recommended draft LLNA performance standards developed to facilitate evaluation of modified LLNA protocols with regard to the traditional LLNA. In response to this *FR* notice, NICEATM received four comments, two of which suggested clarifications to the text. Another recommended that test substances chosen for testing in the various LLNA methods should be pure, with conclusive structures, and should not be mixtures.

The ICCVAM review of the rLLNA, in which only the highest dose is used to assign a positive/negative result for a test substance, was a retrospective evaluation of available LLNA studies with which to compare the outcome of the traditional protocol (in which all doses are considered and any positive result, regardless of concentration, can be used to establish a sensitizing substance). Therefore, although the validation status of the LLNA for testing mixtures is still under review, ICCVAM and NICEATM considered it appropriate to include all available data in the evaluation of the rLLNA.

The fourth commenter addressed the rLLNA in general. The commenter supported the development of performance standards that expedite the validation of new protocols similar to previously validated methods but was disappointed that NICEATM–ICCVAM has chosen to develop performance standards for such a narrow scope of applicability (i.e., modifications of the standard LLNA that involve incorporation of non-radioactive methods of detecting lymphocyte proliferation). The commenter suggested that limited resources available to NICEATM and ICCVAM would be better spent on activities that would have greater impact on

the reduction, refinement, or replacement of animal use, such as evaluating the use of human cell lines or one of the available *in vitro* skin models as a replacement for the LLNA.

ICCVAM considered the comment and concluded that the proposed modifications to the LLNA protocol and expanded applications have significant potential to further reduce and refine animal use. ICCVAM is also interested in *in vitro* models and non-animal approaches for assessing allergic contact dermatitis; however, no *in vitro* replacements for the LLNA have yet been nominated or submitted to ICCVAM for evaluation.

4.3 Public Comments in Response to 73 FR 1360 (January 8, 2008): Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments

NICEATM requested public comments on the draft BRDs, draft ICCVAM test recommendations, draft test method protocols, and revised draft LLNA performance standards for an international independent scientific peer review panel meeting to evaluate modifications and new applications for the LLNA. NICEATM received six comments in response to this *FR* notice. Four commenters focused on the traditional LLNA and two commenters provided comments specific to the rLLNA.

One commenter agreed with ICCVAM's recommendation of the rLLNA for hazard identification purposes, noting that Kimber et al. (2006) did not propose a 10% concentration threshold as the absolute cutoff for defining non-sensitizing chemicals. Gerberick et al. (2005) showed that for some compounds tested the highest concentration was at least 20% and did not induce a positive response at any concentration tested; these compounds were categorized as non-sensitizing. Cockshott et al. (2006) reported that a negative result obtained with the highest concentration tested at 10% would be considered a valid result if the positive control, a mild to moderate sensitizer, gave a positive response (i.e., a chemical that is negative at a top concentration of 10% does not represent a significant human sensitization hazard). This is similar to the definition of a non-sensitizing chemical in the Guinea Pig Maximization Test (GPMT) or Buehler Test as one that induces responses lower than 30% or 15%, respectively. Therefore, if a chemical elicits positive responses in 20% or 25% of the test animals in a GPMT, it would be considered a non-sensitizer from a regulatory perspective.

ICCVAM and the Panel agreed that the maximum applied dose for the rLLNA should be based on the absence of overt systemic toxicity and/or excessive local irritation. The available data did not support establishment of a uniform concentration threshold for the maximum concentration to be tested.

Another commenter's response referred first to the April 2007 ESAC statement:

> "…supporting the use of the rLLNA 'within tiered-testing strategies to reliably distinguish between chemicals that are skin sensitisers and non-sensitisers,' thereby reducing animal use by as much as 50%. In spite of the ESAC recommendation, ICCVAM conducted its own data call-in and data review. The reviewed database is comprehensive and contains a broad cross-section of the chemical universe. The performance characteristics were all above 95% (false negative and positive rates are very low or zero). Even though this additional review was largely unnecessary, [the

commenter was] pleased that ICCVAM's draft recommendations concluded favorably for the rLLNA procedure…"

The commenter urged the Panel to concur. As reflected in the Independent Scientific Review Panel Assessment (**Appendix F**), the Panel generally agreed with ICCVAM's test method recommendations for the rLLNA, which have been updated to reflect comments from the Panel, SACATM, and the public.

4.4 Public Comments in Response to 73 FR 29136 (May 20, 2008): Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments

NICEATM requested submission of written public comments on the Independent Scientific Peer Review Panel Assessment. No comments were received in response to this *FR* notice.

4.5 Public and SACATM Comments: SACATM Meeting on June 18-19, 2008

The June 18-19, 2008, SACATM meeting included a discussion of the ICCVAM review of the rLLNA test method.

There were no public comments specific to the rLLNA.

One SACATM member concurred with the recommendation that the rLLNA protocol should discuss how to determine the maximum dose if only a single dose is to be used in a screening process. An investigator must be able to define excessive irritation; otherwise, the testing may produce a bell-shaped response curve. In response to this comment and the Panel's recommendation, ICCVAM added to the updated LLNA test method protocol specific guidance on how to determine the maximum concentration to be tested so as to avoid overt systemic toxicity and/or excessive local irritation (**Appendix B, Annex III**).

Another SACATM member suggested that the rLLNA appeared favorable because 100% (153/153) of the non-sensitizing agents and 98.1% (312/318) of the sensitizing agents were correctly predicted. ICCVAM agrees that this high level of agreement between the traditional LLNA and the rLLNA supports routine use of the rLLNA as recommended by ICCVAM.